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Influence of Environmental Factors on the Production of MIB and Geosmin Metabolites by Bacteria in a Eutrophic Reservoir

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Key Points:
- Known taste and-odor-causing bacteria were identified using the 16S rRNA gene sequencing methodology
- Peak spring discharges and nitrogen inputs are important drivers on the distribution of bacteria and two taste-and-odor compounds: MIB and GSM
- Algaecide treatment severely impacted GSM-causing cyanobacteria but was less effective against MIB-producing actinobacteria

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Abstract Occurrences of odorous bacterial metabolites, 2-methylisoborneol (MIB) and geosmin (GSM), in drinking water supply reservoirs are considered as a nuisance by the water industry and a source of complaints from customers. In Eagle Creek Reservoir, routine monitoring programs of MIB and GSM highlight intense odorous outbreaks during the spring season when high inflow discharges occur. Cyanobacteria have always been assumed to be source of these metabolites even if no known producers are present in raw water. A copper-based algaecide is often used to terminate the metabolite production and the algal growth in the reservoir. The current study was designed to investigate and identify other biological sources involved in the biosynthesis of MIB and GSM metabolites as well as environmental factors that could be important triggers for the growth of bacterial producers. The community structure of the bacterioplankton was determined using a 16S rRNA gene sequencing technique, which showed that not only Cyanobacteria but Actinobacteria also were involved in the reservoir internal production. *Planktothrix* species was identified as the main source of GSM ($p < 0.001$) while *Streptomyces* (Actinobacteria) was very likely responsible of MIB ($p < 0.01$). Application of an algaecide disrupted GSM and the growth of *Planktothrix* but was less effective against MIB and *Streptomyces*. Statistical analyses revealed that MIB- and GSM-causing bacteria were found abundant when the water was enriched with nitrogen, temperature cooler, and the water column mixed.

1. Introduction

Continental freshwater systems have received increasing scientific attention over the past decades as water quality deterioration and cyanobacterial bloom activity have been linked to eutrophication and global warming (Paerl et al., 2001; Paerl & Huisman, 2009; Shatwell et al., 2008). When excessive nutrient is supplied to surface waters and temperature is optimal for growth, Cyanobacteria rapidly form massive water blooms (Dokulil & Teubner, 2000) that produce small dissolved organic compounds frequently involved in ecological (Christoffersen, 1996; Miguéns & Valério, 2015), economical (Dodds et al., 2009; Steffensen, 2008), and health issues (Carmichael, 2001). Such compounds can also support the growth of heterotrophic bacteria and shape the structure of the bacterioplankton community (Eiler & Bertilsson, 2004; Louati et al., 2015). Occurrences of off-flavor compounds synthetized by aquatic bacteria are a nuisance in source water systems, and numerous episodes of taste-and-odor (T&O) compounds have been reported worldwide (Juttner & Watson, 2007; Krishnani et al., 2008).

In natural environments, two terpenoids, geosmin (GSM; trans-1,10-dimethyl-trans-9-decalol) and 2-methylisoborneol (MIB), are the main metabolites causing T&O problems in drinking water (Lanciotti et al., 2003; Ma et al., 2013; Watson et al., 2008; Westerhoff et al., 2005). They impart an earthy (GSM) and musty (MIB) taint to the water (Izaguirre & Taylor, 2004) and to fish in aquaculture (Klausen et al., 2005; Robin et al., 2006). Due to their lipophilic properties, both MIB and GSM easily cross the gills and guts of fish, causing longer depuration or purging times for the removal of the earthy/moldy flavors accumulated in fish flesh prior to commercialization (Burr et al., 2012; Davidson et al., 2014; Howgate, 2004; Reineccius, 1991). Each compound exists as (+) and (−) enantiomers, but biological sources produce the (−) stereoisomer (Krasner, 1988) which is 10 times more potent than its (+) counterpart (Juttner & Watson, 2007). Both MIB and GSM are potent odorous metabolites and have very low odor threshold concentrations (at parts per trillion levels (or ng/L)) that can be detected by a human's olfactory senses (Peter & Von Gunten, 2007). Drinking water quality, and thus the value of that water, is impacted by frequent

occurrences of MIB and GSM in water supplies (Davies et al., 2004; Srinivasan & Sorial, 2011). The tertiary alcohol structure of both GSM and MIB renders them extremely resistant to oxidation processes commonly used in water purification. Low concentrations tend to persist in finished water as conventional water treatment processes such as air stripping (Terashima, 1988), dissolved air flotation (Hargesheimer & Watson, 1996), flocculation/sedimentation/sand filter (Hargesheimer & Watson, 1996), oxidation with chlorine (Cl_2), chloramines and chlorine dioxide (ClO_2; M. McGuire, 1999; Nerenberg et al., 2000), or potassium permanganate ($KMnO_4$; M. McGuire, 1999) fail to remove them entirely. Ozone (O_3) remains the strongest oxidant to remove efficiently MIB and GSM, but their oxidation can generate byproducts such as low molecular weight ketones that also have odorous properties (Lundgren et al., 1988; M. J. Mcguire & Gaston, 1988). Besides the offensive odorous properties in source, recreational, and drinking waters, T&O compounds currently have no regulations in the United States because they are associated with no known adverse effects on human health (Dionigi et al., 1993). Therefore, the U.S. Environmental Protection Agency (USEPA) has defined no maximum concentration level or maximum concentration level goal for MIB and GSM in drinking water.

The most important source of GSM and MIB in surface waters is bacteria (Juttner & Watson, 2007; Watson, 2003). Both compounds are secondary metabolites synthetized through the isoprenoid pathway (Bentley & Meganathan, 1981), but their biological functions have not been elucidated. Trace concentrations of various odorous metabolites produced by bacteria may change the organoleptic properties of water and act as chemical attractants or repellents in the aquatic food web for invertebrates, fish, and humans (Höckelmann et al., 2004; Juttner & Watson, 2007; Watson et al., 2007). In freshwater environments, Cyanobacteria have been known as the major producers of odorants (Juttner & Watson, 2007; Watson, 2010; Watson et al., 2008). Off-flavor compounds, such as MIB and GSM, which deteriorate the quality of water, are often associated with seasonal blooms of *Oscillatoria*, *Anabaena flos-aquae*, *Planktothrix*, and *Microcystis aeruginosa* (Hayes & Burch, 1989; Li et al., 2007; Su et al., 2015), where decaying blooms can release many odorous metabolites (Ma et al., 2013; Smith et al., 2008) and other bioactive compounds like cyanotoxins (Smith et al., 2008). Actinobacteria were the first identified organisms as MIB and GSM producers (Gerber, 1979; Gerber & Lechevalier, 1965; Juttner, 1990; B. Zaitlin & Watson, 2006) and are very frequently found in limnetic systems (Glöckner et al., 2000; Methé & Zehr, 1999; Van der Gucht et al., 2005) and in bottom sediments (Boucher et al., 2006; Hahn et al., 2003), where they play a significant role in organic matter degradation (Jiang & Xu, 1996; Johnston & Cross, 1976; Beryl Zaitlin et al., 2003). Other organisms, such as Myxobacteria belonging to δ-Proteobacteria, also have the ability to synthesize GSM and MIB (Dickschat et al., 2005; Dickschat et al., 2007).

The environmental factors triggering the synthesis of GSM by Actinobacteria were studied by Wood et al. (1983). Some of the relevant factors were elevated nutrient levels in water, aerobic conditions, and accumulation of sediment in the reservoir. The importance of nitrogen in the synthesis of GSM by Actinobacteria was later confirmed (Lind & Katzif, 1988). In the cyanobacterium *Fischerella muscicola*, Wu and Jüttner (1988) showed that GSM was indifferently obtained under aerobic or anaerobic conditions and that GSM production was minimal at the optimal growth temperature but maximal at the lowest and highest temperature ranges. The influence of light and nutrient (N and P) on the synthesis of GSM by *Oscillatoria brevis* was demonstrated to have no direct effect (Naes et al., 1985). Instead, it was concluded that GSM detections were the result of increased algal biomasses due to excess nutrient conditions rather than increased production rates (Wnorowski, 1992).

Every year, Eagle Creek Reservoir providing drinking water to the city of Indianapolis, IN experiences redundant occurrences of MIB and GSM that taint its source water between the fall and the spring seasons. Depending on the intensity of concentrations in raw water, the local water company encountered difficulties at removing efficiently MIB and GSM. Because of the numerous phone calls received from customers complaining about the earthy or musty odors in their tap water or while showering, water utility authorities have begun a monitoring program of MIB and GSM in both the reservoir and the treatment plant in the early 2000s. Alternatively, the reservoir was treated, once or twice a year, with a copper-based algaecide as an ultimate solution to curtail T&O events. Copper is an essential metal, and aquatic organisms have developed strategies to regulate intracellular concentrations (Phillips & Rainbow, 1989). Excessive copper can either be excreted out of organisms (Vijayram & Geraldine, 1996) or sequestered internally (Depledge & Rainbow, 1990). For decades, copper-based algaecides have been used to control algal growth in aquatic

systems (Mastin & Rodgers, 2000). In water, as copper sulfate dissociates into copper and sulfate ions, copper becomes highly bioavailable, and its ionic form is quite toxic to aquatic invertebrates and fish (Closson & Paul, 2014; Pickering et al., 1977; Winner & Farrell, 1976). To increase their effectiveness, newer copper-based algaecides were designed to reduce the copper toxicity to nontarget organisms. When chelated, copper is less toxic to fish, but the inclusion of surfactants to the formulation increases its toxicity (Closson & Paul, 2014). Copper-based algaecides are known to be more effective on Cyanobacteria rather than eukaryotic algae (Calomeni et al., 2014). High biomasses of phytoplanktic algae can even mitigate the copper toxicity to nontarget organisms (Bishop et al., 2018). However, copper-based algaecides rapidly lyse cyanobacterial cells that release their metabolites, such as all cyanotoxins and T&O compounds to the water (Hudnell, 2010). Recurrent algaecide treatments have also been shown to generate spontaneously copper-resistant mutants in *M. aeruginosa*, which may lead to complications for future applications (García-Villada et al., 2004).

In Eagle Creek Reservoir, algaecide products were commonly sprayed in the upper basin and covered approximately one third of the reservoir area. Located right after the mouth of the main tributary, this area is known to be critical for the phytoplankton's growth in the reservoir. Algaecide applications generally caused a few fish kills of fathead minnows (*Pimephales promelas*), wipe the algae for a few days, and terminate the MIB and GSM signals in the raw source water. As MIB and GSM in the reservoir have always been assumed to be produced by Cyanobacteria, a monitoring program of the reservoir phytoplankton was created in 2008. Enumeration and identification with an optical microscope showed that *Planktothrix rubescens* (winter/spring) and *Planktothrix agardhii* (fall) were abundant during the seasonal GSM occurrences whereas *Pseudanabaena catenata* matched MIB occurrences during the spring periods (Tedesco & Clercin, 2010). Although GSM detections were well correlated to the presence of *Planktothrix* species in the reservoir, peaks of MIB were not always correlated to *Pseudanabaena*. Asynchronicity between the known producer and high detections of MIB was first assumed, but very often, intense outbreaks of MIB occurred while *Pseudanabaena* was completely absent. Environmental parameters such as water temperature and nutrient availability play a key role in the seasonal succession of the reservoir bacterial communities. The main objective of the current study is to determine which environmental triggers are important for the growth of known MIB- and GSM-causing bacteria. As highest detections of MIB and GSM are generally detected during the spring when high inflow discharges occur, the influence of nitrogen and water temperature will be tested as important drivers of the bacterioplankton leading to the in situ production of T&O compounds. Subsequently, we will also try to identify potential producers of MIB during the 2013 outbreak when known cyanobacterial taxa are missing and determine if the reservoir hydrology is also critical.

2. Materials and Methods

2.1. Study Site

Eagle Creek Reservoir (39°51′20″N, 86°17′39″W), located in central Indiana (Figure 1), receives drainage from 419.6 km^2 of the Eagle Creek Watershed (HUC 05120201120) and has a surface area of 5.7 km^2. The reservoir was constructed in 1967 to provide flood control and then drinking water for the city of Indianapolis and surrounding communities. The maximum depth ranges from about 11 to 13 m, with the deepest areas located in the southern basin, near the dam. Eagle Creek Reservoir is a small, dimictic, and eutrophic water body with seasonal thermal stratification from June to September. Reservoir mixings usually occur in April/May and October each year. The mean annual discharge of Eagle Creek, the main tributary, is 35.74 m^3/s with maxima recorded between April and June. The calculated residence time of the reservoir is 39.5 days.

2.2. Sample Collection and Processing

Water samples were collected on a biweekly basis from mid-May to end of October 2013 near the dam where the strongest water column stratifications occur (Figure 1). Discrete water samples were collected with a vertical Van Dorn sampler at four different depths corresponding to subsurface (0 m), summer epilimnion (3 m), metalimnion (6 m), and hypolimnion (9–10 m), that is, 1 m above the water-sediment interface. After collection, all discrete water column samples (0, 3, 6, and 10 m) were put on ice in autoclaved 1-L high-density polyethylene brown bottles and filtered in the lab through 0.22-μm mesh size pores on a

Figure 1. Sampling site location (dot) on Eagle Creek Reservoir. KEYE = Eagle Creek Airpark (square) where weather data were retrieved.

sterile glass filtration unit, then frozen for storage in 15-mL Falcon tubes. Samples were later shipped to Illumina, Inc., San Diego, CA, USA, for analysis on frozen filters to determine the bacterial community assemblages using 16S rRNA gene sequencing.

2.3. In Situ Measurements

The photic zone was measured by the mean of a Secchi disk (SD), and the euphotic depth (Z_{eu}, in meters) was estimated from Secchi disk reading (Z_{SD}) using the relationship $Z_{eu} = 2.7 \times Z_{SD}$ (Tedesco & Clercin, 2010). Transmission of photosynthetically active radiation (μmol·photons·m^{-2}·s^{-1} from 400 to 700 nm) was measured at 50-cm intervals from just below the surface down to a depth of 1% incident photosynthetically active radiation with a LI-192SA Underwater Quantum Sensor (LI-COR Inc., Lincoln, NE, USA). Prior to water collection, a submersible multiparameter V2-6600 YSI probe (YSI, Inc., Yellow Spring, OH, USA) was deployed in order to characterize the water column at meter intervals from the water surface down to the bottom. Measured parameters were water temperature (Temp; °C), conductivity (COND; μS/cm), total dissolved solids (TDS; g/L), dissolved oxygen (DO; mg/L), pH (s.u.), oxidation reduction potential (ORP; mV), and chlorophyll and phycocyanin fluorescence (RFUs; relative fluorescence units). The intensity of the reservoir thermal stratification was assessed by calculating the relative thermal resistance to mixing (RTRM) between adjacent layers (1-m increment) within the water column. RTRM values were computed from temperature profile data using the relation (Wetzel, 2001): $\psi = (\rho_{z2} - \rho_{z1})/(\rho_4 - \rho_5)$, where ψ is the RTRM value (dimensionless), ρ_{z1} and ρ_{z2} are water densities at depths z_1 and z_2, respectively (kg/m^3), and ρ_4 and ρ_5 are water densities at 4 and 5 °C, respectively. Greater density differences between water layers are highlighted by higher RTRM values. Boundaries of the metalimnion are identified by RTRM 30, while the maximum value of RTRM identifies the depth of the thermocline. When RTRM values exceed 80, they are characterized as being "strongly stratified" (Vallentyne, 1957).

2.4. Nutrients and T&O Compounds

Aliquots of the water sample were stored in 1-L white high-density polyethylene bottles for nutrient analyses. Samples for MIB and GSM analysis were stored in brown amber glass jars with no headspace or bubbles to avoid the volatilization of these compounds. All samples were stored on ice for transport to the laboratory. Inorganic nitrogen forms (nitrate and nitrite) were measured by ion chromatography Dionex DX-500 using the EPA 300.0 method (USEPA, 1993a). Total Kjeldahl Nitrogen was determined by

digestion, followed by ammonia determination by ion selective electrode (USEPA, 1993b). Total P was measured by ascorbic acid colorimetric method (USEPA, 1974). GSM and MIB concentrations in water were quantified by head-space solid-phase microextraction combined with gas chromatography-mass spectrometry to analyze the volatile metabolites MIB and GSM according the Standard Method SM 6040D (APHA, 2000).

2.5. Enzyme-Linked Immunosorbent Assay

Water sample aliquots were put in acid washed 125-ml clear glass for microcystin analysis using the enzyme-linked immunosorbent assay analytical method, which is sensitive for low levels of microcystins (Pyo et al., 2005) in raw water; the limit of detection of this method is 0.15 μg/L (ppb). Three freeze/thaw cycles followed by sonication (15 min at 40 kHz) were used to optimize the extraction of cyanotoxins. An aliquot (1 ml) of each sample was used for total microcystins analysis using competitive enzyme-linked immunosorbent assay kits (Fischer et al., 2001) targeting the nonproteinogenic amino acid (ADDA) found in cyanobacterial toxins following the protocols supplied by the manufacturer (Abraxis LLC., PA, USA). All assays were performed in duplicate. For statistical and graphical purposes, half of the limit of detection value was used to represent nondetected concentrations of microcystins in water (Croghan & Egeghy, 2003).

2.6. Hydrology and Weather Data

Stream discharge data from Eagle Creek, the main tributary of the reservoir, was recorded by the USGS super gage (USGS 03353200), located upstream from the water body in Zionsville, IN, USA. Weather data were recorded by Eagle Creek Airport (Figure 1, KEYE), adjacent to the reservoir.

2.7. Bacterial Community Identification

2.7.1. DNA Isolation and Library Preparation

DNA was isolated from collected water samples using Meta-G-Nome™ DNA isolation kits. From each sample, approximately 700 ng of DNA were extracted and then prepared following the 16S library preparation protocol and the Nextera® XT DNA index kit from Illumina.

2.7.2. Sequencing

Samples were loaded onto a MiSeq v.3 reagent cartridge and then onto the next-generation sequencing instrument. Two 300-bp paired-end sequencing runs were performed, and the resulting sequence reads were equally distributed across samples, demonstrating uniform coverage. Targeted sequences were the 16S rRNA V3 and V4 regions using 5′ TCGTCGGCAGCGTCAGATGTGT ATAAGAGACAGCCTACGGGNGGCWGCAG and 5′ GTCTCGTGGGCTCGGAGATGTGT ATAAGAGACAGGACTACHVGGGTATCTAATCC as forward and reverse primers, respectively. The 16S rRNA gene sequencing provides reliable information on the taxonomic composition and the phylogenetic structure of natural bacterial communities.

2.7.3. Genomic Data Analysis

Generated sequencing data sets were analyzed with MiSeq Reporter software from Illumina BaseSpace platform. The 16S Metagenomics App provides summary reports for each sample. Detailed results from Eagle Creek Reservoir are available to public view by searching for "MiSeq v3: Nextera XT Metagenomics Water Samples" or by using the following link: basespace.illumina.com/projects/9954952/MiSeq-v3-Nextera-XT-Metagenomics-Water-Samples (last accessed: November 2018).

2.7.4. Optical Microscope Identification

Cyanobacterial taxa were identified using a Nikon Labophot 2 microscope at 200X and 400X magnification. Observations of major taxa in water samples including *P. agardhii* and *P. rubescens*, *Microcoleus* sp., *Pseudanabaena limnetica* and *P. catenata*, *Aphanizomenon flos-aquae*, *Dolichospermum* spp., *M. aeruginosa*, and *Cylindrospermopsis raciborskii* thus validated results obtained from the 16S analysis. However, the visual observation could not identify picocyanobacteria such as *Prochlorococcus* sp. or any actinobacterial taxa whence identification relied solely on 16S analysis.

2.8. Statistical Analysis

The 16S data set along with physical and chemical parameters collected during the sampling campaign were analyzed with PAST 3.1 software (Hammer et al., 2001). For the need of the analyses, we combined all species belonging to the same genus as Operational Taxonomic Units. We used Spearman's ρ correlations to assess potential links between bacterial genera and odorous metabolite concentrations and canonical correspondence analysis (CCA) to extract major gradients among all physicochemical parameters that could

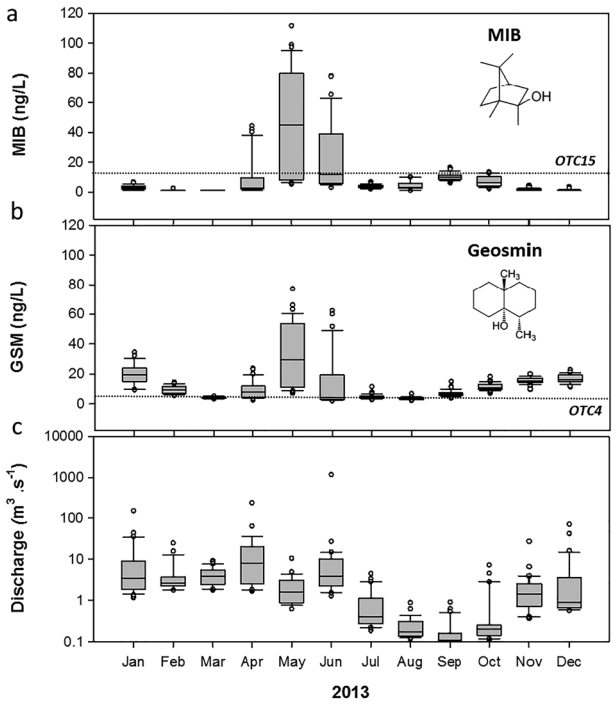

Figure 2. Monthly concentrations of (a) 2-methylisoborneol (MIB), (b) geosmin (GSM), and (c) Eagle Creek discharges for the year 2013 (note log scale). OTC = odor threshold concentrations (dotted lines) after Peter and Von Gunten (2007).

trigger the growth of bacteria and the production of metabolites. Using SigmaPlot 13, box and whisker plots were used to display the distributions of the data with the central rectangle spanning the first quartile to the third quartile. The median value is represented by the segment inside the rectangle. Whiskers show the tenth and ninetieth percentiles, and all extremely low or high outlier beyond these percentiles are represented by dots.

3. Results and Discussion

3.1. Metabolite Detections in Raw Water

On a routine basis, the local water company analyzes Eagle Creek Reservoir's raw water samples for odorous compound detections at several locations: at the dam, at the water intake, and before the entry of the water treatment plant. Two or three analyses are run weekly, and the sampling frequency is usually augmented when either MIB or GSM levels exceed 10 ng/L. In 2013, a total of 359 water samples were analyzed, with an annual average of 10.8 and 12.6 ng/L for MIB and GSM, respectively (Figure 2). Highest detections are commonly found between the months of April and June. Maximal values of 111.8 (MIB) and 77.3 ng/L (GSM) were recorded on 22 and 31 May 2013, respectively. It is noticeable that MIB concentrations exceed the odor threshold value of 10 ng/L seasonally during the spring and early fall while GSM detections are always above 4 ng/L but show minima throughout the summer months. GSM exceeds its odor threshold concentration 100% of the times when sampled from October to February and during the month of May outbreaks.

3.2. T&O Producers

3.2.1. Distribution of Bacteria

Genetic data for the 2013 sampling campaign illustrate Cyanobacteria as a critical part of the bacterioplankton community, but there are other orders distributed with depth (Figure 3). Proteobacteria are the second largest group and include a wide range of organisms found commonly, but none of them are known producers of T&O compounds. Actinobacteria are known producers of T&O compounds and are found in significant abundance in the water column at all depths. On average, Cyanobacteria represent 36% of the bacterioplankton community, followed by Proteobacteria (25%), Actinobacteria (7%), and all other groups individually lower than 5%. Overall, Cyanobacteria tend to be more abundant in the top layers of the water column and peak at the depth of 3 m, whereas Proteobacteria tend to show higher densities near the bottom. The distribution of Actinobacteria seems to be fairly mixed within the water column (Figure 3).

3.2.2. Identification of T&O Producers

The 16S rRNA gene sequencing technique identifies 32 bacterial genera capable of producing either MIB and GSM, or both compounds: 18 genera belonging to Cyanobacteria with 10 of them as potential producers and 14 genera from Actinobacteria with only three of them as potential producers. No Myxobacteria were detected in the samples (Table 1). Spearman's ρ correlations highlight significant correlations between Cyanobacteria and GSM and between some Actinobacteria and MIB. Spearman correlations show that the production of GSM in Eagle Creek Reservoir is linked to several genera from the Oscillatoriales and Nostocales orders (Kutovaya & Watson, 2014; Suurnäkki et al., 2015; Taylor et al., 2006) but not positively correlated with any statistical significance to actinobacterial genera. The link between GSM occurrences and the presence of Cyanobacteria, especially *Planktothrix* species ($p < 0.001$), in the reservoir is in concordance with past observations on the microscope and validates this hypothesis. Conversely, the MIB production is linked to some genera from Actinomycetales but not positively correlated with any cyanobacterial genera (Table 1). Actinobacteria are well-known T&O producers usually associated with soil environments (Juttner, 1990), bottom sediments (Sugiura & Nakano, 2000), or suspended sediments (Jensen et al., 1994), whereas many species have not been documented yet about their potential capability to produce volatile odorous compounds (Table 1). Our results strongly suggest that MIB production in Eagle Creek Reservoir

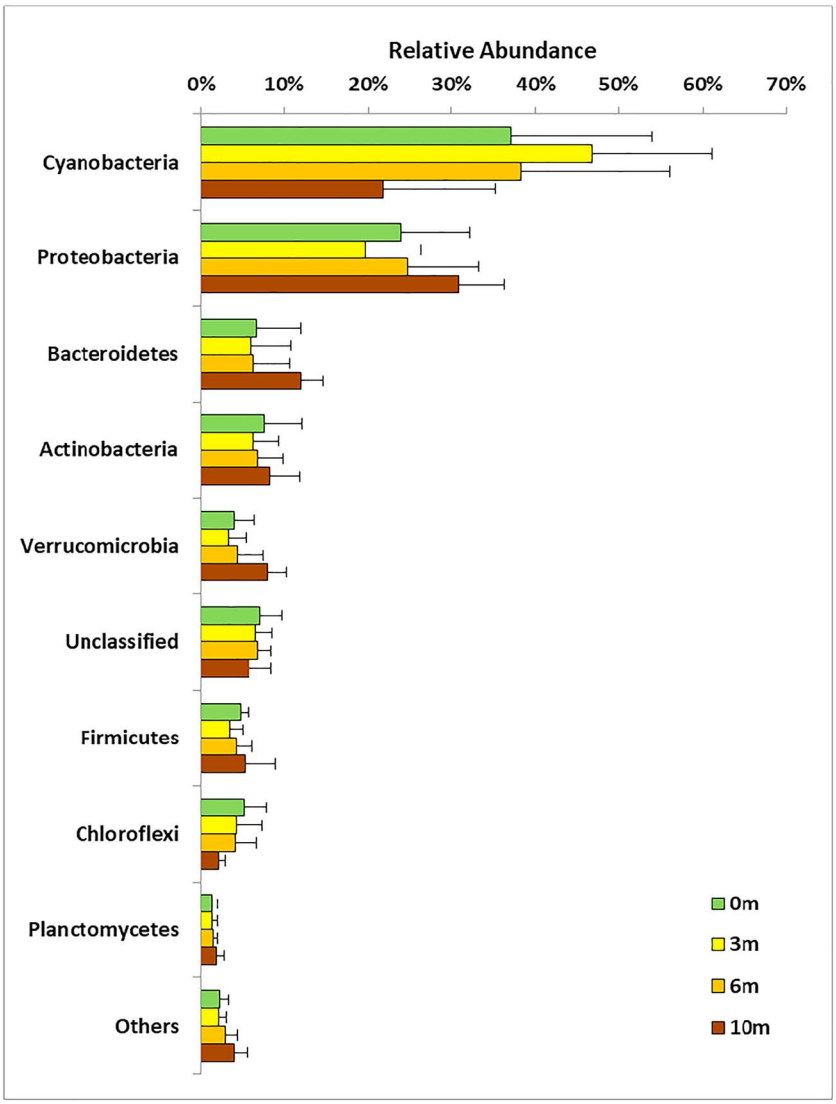

Figure 3. Average relative abundance of bacterial phyla during 2013 campaign at the four discrete sampling depths.

is unlikely linked to Cyanobacteria but rather to Actinobacteria. Nevertheless, the cyanobacterial contribution in the production of MIB is not totally excluded (Su et al., 2015). Thanks to the genetic analysis, we can confirm that some Actinobacteria like *Streptomyces* species ($p < 0.01$) are involved in the production of MIB in the reservoir and fill the gap when no cyanobacterial producers are observed in the source water.

3.3. Hydrology and T&O Outbreaks

3.3.1. Reservoir Hydrology

Past observations of T&O events in Eagle Creek Reservoir suggest that hydrological drivers are key parameters on the production of MIB and GSM metabolites by different bacterial groups. These findings have direct implications on the forecasting and the management of off-flavor occurrences in source waters and the optimization of MIB and GSM removal. Eagle Creek Reservoir is a dimictic water body and receives most of its water during the spring: in April, from snow melt, and in May/June thanks to rainfalls and thunderstorms (Table 2). Peak discharges bring terrestrial materials from soil erosion in the upstream watershed and could introduce Actinobacteria into the reservoir (Beryl Zaitlin et al., 2003). Eagle Creek Reservoir usually stratifies from mid-June to September each year. Then, the second mixing period begins in late September to early October with the return of dominant winds from the North.

Table 1
Spearman's ρ Correlations Between Potential Taste-and-Odor Compound Producers Found in Eagle Creek Reservoir (ECR) With Measured Concentrations of 2-Methylisoborneol (MIB) and Geosmin (GSM)

Phylum	Order	Genera	MIB	GSM	Habitat	Metabolites	References
Cyanobacteria	Chroococcales	*Chroococcus*	**−0.48***	0.07	Planktic	—	—
		Cyanobacterium	−0.27	0.37	Planktic	—	—
		Microcystis	**−0.58****	−0.18	Planktic	—	—
		Prochlorococcus	**−0.59****	**−0.48****	Planktic	—	—
		Snowella	−0.23	0.16	Planktic	—	—
	Oscillatoriales	*Microcoleus*	−0.17	**0.45***	Planktic	GSM	Izaguirre and Taylor (1995)
		Oscillatoria	**−0.57****	−0.07	Benthic	MIB, GSM	Izaguirre et al. (1983), Suurnäkki et al. (2015)
		Phormidium	−0.15	**0.42***	Benthic	GSM	Izaguirre and Taylor (1995)
		Planktothrix	−0.17	**0.53****	Planktic	MIB, GSM	Kutovaya and Watson (2014), Su et al. (2015)
	Pseudanabaenales	*Leptolyngbya*	**−0.41***	0.06	Epiphytic	MIB, GSM	Wang et al. (2015), Watson et al. (2016)
		Limnothrix	**−0.54****	0.07	Planktic	—	—
		Prochlorothrix	**−0.46***	−0.01	Planktic	—	—
		Pseudanabaena	**−0.59****	0.04	Planktic	MIB	Zimba et al. (1999)
	Nostocales	*Aphanizomenon*	**−0.60****	−0.16	Planktic	GSM	Kutovaya and Watson (2014), Suurnäkki et al. (2015)
		Calothrix	**−0.31**	**0.32**	Epiphytic	GSM	Kutovaya and Watson (2014), Suurnäkki et al. (2015)
		Cylindrospermopsis	**−0.45***	−0.02	Planktic	—	—
		Dolichospermum	**−0.75****	**−0.37**	Planktic	GSM	Watson et al. (2016)
		Nostoc	−0.22	**0.44***	Benthic	GSM	Taylor et al. (2006)
Actinobacteria	Actinomycetales	*Arcanobacterium*	0.27	−0.15	Soil	—	—
		Cryobacterium	**0.44***	−0.05	Soil	—	—
		Demequina	**0.33**	−0.24	Soil	—	—
		Georgenia	**0.40***	−0.15	Soil	—	—
		Mycobacterium	0.10	−0.20	Soil	—	—
		Nocardia	0.15	0.17	Soil	MIB, GSM	Zaitlin and Watson (2006)
		Rhodococcus	−0.06	**−0.32**	Soil	—	—
		Saccharomonospora	**0.45***	−0.11	Soil	—	—
		Saccharopolyspora	−0.24	−0.13	Soil	MIB, GSM	Komatsu et al. (2008); Watson et al. (2016)
		Sanguibacter	**0.41***	−0.06	Soil	—	—
		Streptomyces	**0.42***	−0.13	Soil	MIB, GSM	Saadoun et al. (1997)
		Streptosporangium	0.23	0.08	Soil	—	
	Acidimicrobiales	*Acidimicrobium*	−0.13	−0.01	—	—	—
		Acidithiobacillus	0.08	0.14	—	—	—

Note. Correlations with strong statistical significance are in bold $p<0.05$.
*$p<0.01$. **$p<0.001$.

3.3.2. Influence of Inflow Discharges

The maxima of T&O compounds were recorded 37 days after a peak discharge of 236.7 m^3/s that occurred on 29 April 2013. A cross-correlation analysis of stream discharges versus metabolite concentrations recorded between 1 April and 30 June 2013 shows a positive correlation of both MIB and GSM peaks with the inflow after a 37-day delay (Table 3). This delay value is closely similar to the reservoir's calculated annual residence time of 39.5 days and supports the hypothesis that inflow discharges as a nitrogen pulse would favor the growth of T&O-causing bacteria in this reservoir. This nitrogen signature also matches the period of the year when farmers apply fertilizers in Eagle Creek watershed. However, during the months of April and May, average monthly retention times were shorter than the annual average, with 1.6 and 14.7 days, respectively (Table 2). This implies that any bacteria would undergo a lag time prior to exponential growth and production

Table 2
Mean Monthly Inflow Discharge (Q, in Cubic Meters per Second) and Mean Residence Time (RT, in Days) of Eagle Creek Reservoir, Year 2013

	Annual	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
N	365	31	28	31	30	31	30	31	31	30	31	30	31
Mean *Q*	52.0	124.7	44.6	42.3	211.4	23.2	72.9	9.2	2.4	1.8	7.5	25.7	58.7
Mean RT	39.5	2.7	7.7	8.1	1.6	14.7	4.7	37.0	139.5	193.3	45.8	13.3	5.8

Table 3
Cross-Correlation Between Off-Flavor Metabolites (2-Methylisoborneol [MIB] and Geosmin [GSM]) Versus Main Tributary Inflow (Q)

MIB			GSM		
Lag (d)	Correlation	*p*	Lag (days)	Correlation	*p*
−40	0.414	0.0493	−40	0.423	0.0443
−39	0.412	0.0454	−39	0.516	0.0099
−38	0.529	0.0065	−38	0.402	0.0461
−37	**0.582**	**0.0018**	**−37**	**0.665**	**0.0002**
−36	0.402	0.0376	−36	0.386	0.0466
−35	0.233	0.2322	−35	0.249	0.2015
...	...	...	...	...	...
−3	−0.107	0.4488	−3	−0.028	0.8457
−2	−0.216	0.1212	−2	−0.141	0.3153
−1	−0.223	0.1047	−1	−0.132	0.3420
0	−0.148	0.2825	0	−0.077	0.5781

Note. Lags are expressed in days. The significant peak discharge that occurred 37 days before the odorous episode of MIB and GSM observed in the reservoir is highlighted in bold.

of T&O compounds in the reservoir. Temperature plays a major role in bacterial growth but also in the production of metabolites (Usha Kiranmayi et al., 2011). Reservoir water temperatures in April/May are cool (<16.7 °C) and far from optimal growth temperatures for freshwater planktonic Actinobacteria; that is, 25–35 °C (Hahn & Pöckl, 2005). In temperate lakes, freshwater Actinobacteria have lower growth rates and lower optimal growth temperatures (0.34/hr and 28 °C, respectively) compared to subtropical and tropical habitats (0.41 hr and 34 °C) or culture media (0.6/hr and 35 °C; Flowers & Williams, 1977; Hahn & Pöckl, 2005). The observed 37-day delay would provide enough time to support a slower growth rate of Actinobacteria and peak production of T&O metabolites as recorded by the end of May in Eagle Creek Reservoir.

3.3.3. Spatiotemporal Distributions

The distributions of MIB and GSM within the reservoir water column is presented in Figure 4 and compared to the distribution of two well-known GSM-producing Cyanobacteria *Nostoc* (Taylor et al., 2006) and *Planktothrix* (Kutovaya & Watson, 2014) as well as two well-known MIB- and GSM-producing Actinobacteria *Nocardia* (Zaitlin & Watson, 2006) and *Streptomyces* (Saadoun et al., 1997). During the May outbreak, highest concentrations of MIB and GSM compounds are observed in the top layers of the water column despite the fact the water column is fully mixed and turbid. Cool temperatures and low light stimulate the GSM production in Cyanobacteria (Zhang et al., 2009). Throughout the summer, very low detections of each metabolite are found when the reservoir stratifies. A bottom signal of MIB suggests that this metabolite is still being produced by Actinobacteria (Streptomyces; $p < 0.01$) and/or recalcitrant to microbial degradation in the hypolimnion. When the water column mixes again from late September 2013, both MIB and GSM produced near the bottom are resuspended.

3.4. Copper-Based Algaecide Treatment

3.4.1. Algaecide Application

In order to curtail the massive T&O production of May 2013, the local water company decided to apply a Cutrine-Plus®-based algaecide on 3 June 2013, which had an immediate impact on Cyanobacteria. About

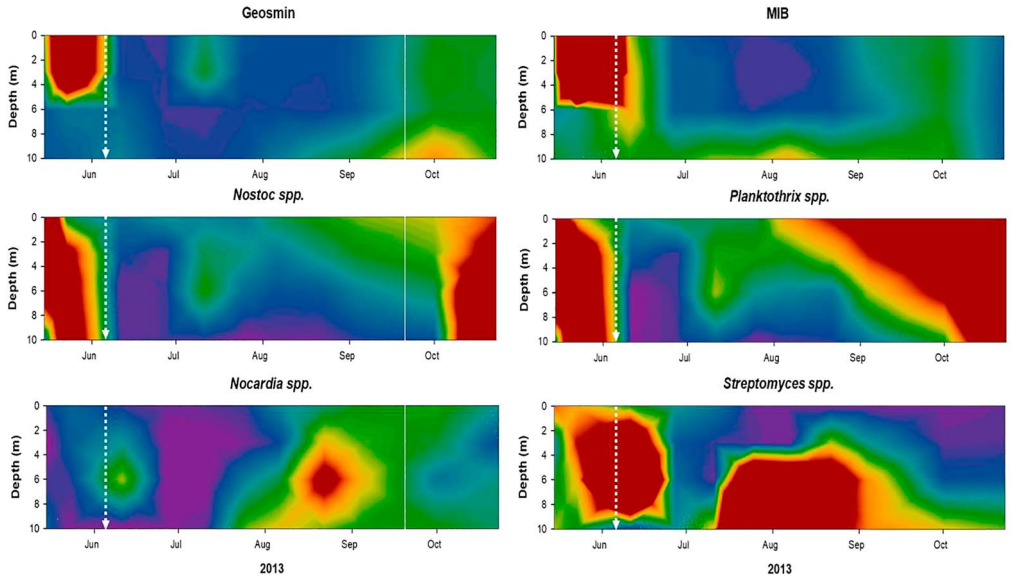

Figure 4. Spatial and temporal distribution of odorous metabolites and main bacterial orders throughout the water column. Top row: GSM and MIB; middle row: Cyanobacteria; bottom row: Actinobacteria; vertical dotted arrow: algaecide treatment date. Warmer colors represent highest concentrations or relative abundances. White arrow indicates timing of the 3 June 2013 algaecide application.

one third of the reservoir corresponding to the upper North basin received the algaecide treatment. A few hours later, the large sludge of organic matter and dead phytoplankton biomass drifted toward the dam, located south of the reservoir and then recovered our sampling site, which was resampled a week later on 11 June 2013. On the treatment day, no major impact on the aquatic life was observed except the death of some fathead minnows (*P. promelas*) that are sensitive to small amounts of copper in water (Welsh et al., 1996).

3.4.2. Impact on Bacterioplankton

The natural decay of *Planktothrix* spring bloom was already underway by the time the water company gets its authorization to apply the algaecide. This may have increased the algaecide effectiveness against Cyanobacteria but had a lesser effect on the Actinobacteria (Figure 4). Though the algaecide addition did disrupt the spring bloom enough to end significantly the GSM production, most Actinomycetales like *Streptomyces* and associated MIB production were not strongly affected by the algaecide treatment. In Eagle Creek Reservoir, species like *Streptomyces nanchangensis*, *Streptomyces roseogilvus*, and *Streptomyces lazureus* did not show any sign of higher abundances after the algaecide treatment application. *Streptomyces* species maintained their densities until mid-June and then began to decline when the water column stratified. The higher resistance of *Streptomyces* is explained not only by its frequent exposure to copper toxicity in sediments but also by its ability to detain copper intracellularly for a few days before a drastic efflux to detoxify cells thanks to the activity of cupric reductases (Albarracín et al., 2008). On 11 June, the continued production of MIB was more diffuse in the water column, but recorded concentrations were lower than 20 ng/L. *Nocardia* species appeared at the depth of 6 m and was very likely associated with the deep production of MIB right after the algaecide application (Figure 4).

3.5. Summer Stratification and T&O Occurrences

3.5.1. Epilimnion

Throughout the summer, the thermal stratification of the water column was strong and influenced the distribution of odorous metabolite-producing bacteria. The water column was stratified from end of June to mid-September, and some GSM production was observed in early July, likely due to the active growth of Nostocalean cyanobacteria such as *Dolichospermum* in the epilimnion, a well-known GSM producer (Watson et al., 2016). The production of GSM was maximal around the depth of 3 m (Figure 4). No other detection of GSM was later found during the summer period.

3.5.2. Hypolimnion

Interestingly, the MIB signal was exclusively recorded in the hypolimnion of the reservoir, between 8 and 10 m, whereas the upper parts of the water column was below detection (Figure 4). The 16S analysis documented the detection of Acidimicrobiales in June and again in late September and Actinomycetales from June up to the end of October near the bottom. Actinobacteria are known to be prevalent and abundant in freshwater bottom sediment (Schrader & Blevins, 1993; Sugiura & Nakano, 2000), but the question of their survival under suboxic or anoxic conditions remains uncertain as abundances decrease with oxygen depletion (Taipale et al., 2009). Here the 16S signature near the bottom simply does not necessarily distinguish between live and dead or senescent cells. Actinobacteria that spread out after the application of an algaecide occupied the whole water column when it was mixed during the month of June. When thermal stratification began to occur from early July, Actinobacteria progressively disappeared from the top layers of the water column and then were found near the bottom. Also noteworthy, the tailing of MIB detections can be observed near the bottom, whereas no GSM was recorded in the hypolimnion. Most of bacterial genera found in the hypolimnetic zone are identified as Actinobacteria, specifically, the anaerobic cellulolytic *Micromonospora* (Leschine et al., 1988), the saprophytic and potentially pathogenic *Mycobacterium* (Kazda, 2010), the humic acid-reducing *Propionibacterium* (Benz et al., 1998), and the alkaline soil indicator *Yonghaparkia* (Yoon et al., 2006). As none of these taxa are documented as potential off-flavor metabolite degraders, the recorded hypolimnetic MIB signal could simply result from cellular release after bacterial breakdown, with MIB remaining nondegraded afterward. MIB concentrations that mimicked this Actinobacteria pattern thus reflect the release of dissolved metabolite into the bottom water after cellular death.

3.6. Influence of Environmental Factors

Reservoir hydrology is a critical driver influencing the spatial distribution of bacterial communities (Šimek et al., 2011). Seasonal mixing and thermal stratification create different habitats for bacteria, each having its

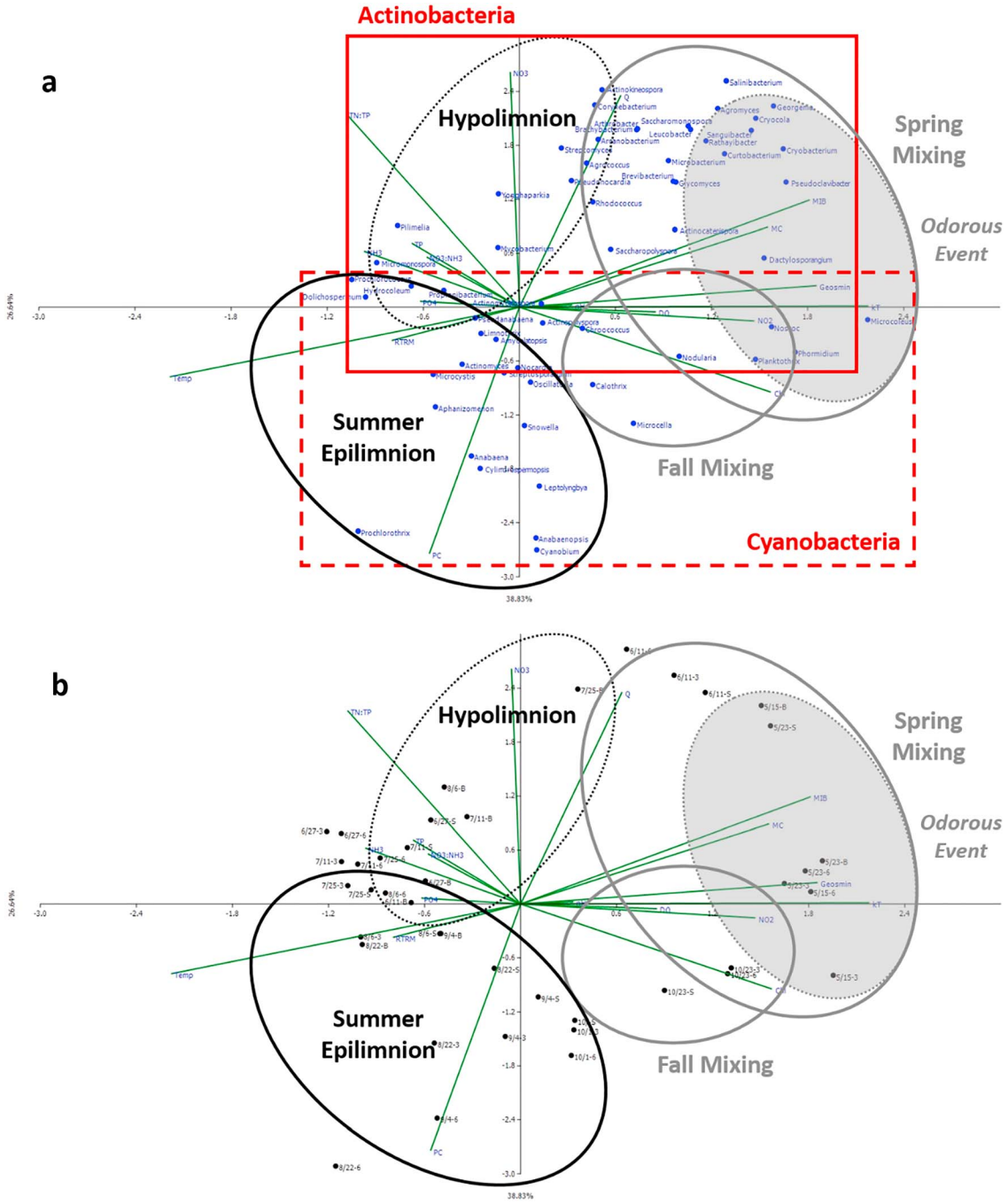

Figure 5. Canonical correspondence analysis showing (a) physical, chemical, and genetic-based microbial data and (b) the distribution of 2013 campaign samples. Ellipses: mixed water column (grey), stratification (black), hypolimnion (black dotted), taste-and-odor event (shaded). Rectangles: Actinobacteria (solid line) and Cyanobacteria (dashed line). Blue dots represent bacterial genera, and vectors are environmental parameters. Black dots are encoded as sampling dates-depths (S = surface; 3 m, 6 m, and B = bottom).

own characteristics in terms of light, pH, temperature, oxygen, or nutrient gradients (Ramsing et al., 1996). To assess the influence of the reservoir hydrological cycle on the seasonal successions of bacterial genera, a CCA was used (Figure 5). This constrained ordination technique extracts major gradients among the multitude of environmental variables measured on the field that would explain the abundances of bacterial genera at a given time of collection. Axes represent linear combinations of all environmental variables maximally projected in a Euclidean space, with axis 1 explaining 38.83% and axis 2, 26.64% of total

variance of the data set. Each axis of the CCA describes separate habitats influenced by gradients of different environmental variables (here physicochemical parameters), which are represented as vectors with arrowheads indicating the direction of the increasing gradients. The dispersion of individual bacterial taxa is ruled by different environmental gradients. For Eagle Creek Reservoir, this multivariate analysis illustrates the seasonal succession of Actinobacteria and Cyanobacteria that are correlated to the hydrological regime. The cloud of dots representing bacteria separates two distinct clusters: with Cyanobacteria in the lower part of the horizontal axis (dashed box) and Actinobacteria in the upper part (full box). Therefore, vectors crossing clusters of dots are more significantly important for these given clusters than distant or opposite ones. In other words, the longest vector lines pointing toward the delineated boxes indicate which physicochemical parameters are favorable for high densities of Actinobacteria and Cyanobacteria or high concentrations of MIB and GSM. Ellipses representing different seasonal conditions are defined according to sampling dates and depths of collection.

3.6.1. Water Temperature and Reservoir Stratification

The horizontal axis of the CCA is mainly driven by temperatures (Figure 5), which translates into summer stratification on the left side of the CCA and, in opposition, reservoir mixing periods on the right side driven by cooler water temperatures. Most cyanobacteria tend to thrive under high water temperatures and strong water column stratification (high RTRM). In the summer epilimnion, Cyanobacteria such as *Aphanizomenon* spp. and *Anabaena* spp., who can potentially produce GSM (Kutovaya & Watson, 2014; Suurnäkki et al., 2015), *C. raciborskii*, *Anabaenopsis elenkinii*, and *Prochlorothrix* sp. also correlate with elevated phycocyanin signals in the late August samples (Figure 5). From late August, occurrences of other Cyanobacteria such as the benthic *Oscillatoria* (Izaguirre et al., 1983; Suurnäkki et al., 2015) and potentially the epiphytic *Leptolyngbya* (Wang et al., 2015) may also have contributed to the production of GSM although individual contributions to the general background signal are often very difficult to assess (Juttner & Watson, 2007). Nonetheless, a few genera are found in more mixed (low RTRM) and turbid waters (high light extinction coefficients; k_T) with cooler temperatures. These latter cyanobacteria, either benthic (*Nostoc*, *Phormidium*, and *Microcoleus*) or pelagic (*Planktothrix*), often co-occur along with higher detections of GSM and chlorophyll *a*.

3.6.2. Discharges and Reservoir Mixing

In opposition to Cyanobacteria, Actinobacteria are strongly correlated to high stream discharges (Q) coupled with elevated nitrate (NO_3) concentrations. During the spring outbreak (Figure 5; grey ellipse), some bacteria belonging to Actinobacteria are more closely related to high MIB detections under conditions closely similar to GSM-producing cyanobacteria. Vectors corresponding to off-flavor metabolites also point out to May samples included in the shaded area, with MIB pointing toward the cloud of Actinobacteria such as *Saccharopolyspora* (Komatsu et al., 2008; Watson et al., 2016) and *Streptomyces* (Saadoun et al., 1997) and GSM pointing toward spring-blooming Cyanobacteria, such as *Microcoleus* (Izaguirre & Taylor, 1995), *Phormidium* (Izaguirre & Taylor, 2004), and *Planktothrix* (Kutovaya & Watson, 2014). This observed pattern grouping Cyanobacteria with GSM and Actinobacteria with MIB confirms the results from the Spearman's *rho* test (Table 1). At the end of the summer as the water column destratifies, occurrences of both MIB and GSM are observed throughout the water column. According to Figure 4, *Nocardia* was present in the top layers of the water column in September as the reservoir turns over. Meanwhile, the mixing-tolerant cyanobacteria *Planktothrix* (in the order Oscillatoriales) dominate the bacterial community. All co-occurring Cyanobacteria are well-known GSM producers, that is, the pelagic *Planktothrix* (Kutovaya & Watson, 2014), the benthic *Calothrix* (Kutovaya & Watson, 2014; Suurnäkki et al., 2015), and *Nostoc* (Taylor et al., 2006), and correlate positively to GSM detections (Table 1). Increasing concentrations of GSM were observed as the fall bloom of *Planktothrix* becomes more intense and severe in October.

3.7. Role of Nitrogen

3.7.1. T&O Compounds

In Midwestern reservoirs, the production of secondary metabolites MIB and GSM is likely to occur when the growth of potential producers is favored by low TN:TP $< 30{:}1$ (by mass) and low $NO_3{:}NH_3$ ratios (Harris et al., 2016). The CCA from Figure 5a shows that elevated abundances of Actinobacteria and Cyanobacteria are correlated to both low TN:TP and $NO_3{:}NH_3$ ratios. Peaks of MIB and GSM occurred during the spring when TN:TP and $NO_3{:}NH_3$ ratios were lower than 12 and 47, respectively (supporting information S7), which is in concordance with Harris's results (Harris et al., 2016). A study in an

Table 4
Correlation Between Inorganic Nitrogen (Nitrite, NO_2^-; Nitrate, NO_3^-, and Ammonia, NH_3) and Bacteria

Phylum	Order	Genera	NO_2^-	NO_3^-	NH_3
Cyanobacteria	Chroococcales	*Chroococcus*	0.25	0.02	**−0.37**
		Cyanobacterium	−0.05	**−0.53****	−0.29
		Microcystis	−0.11	−0.23	−0.27
		Prochlorococcus	−0.20	0.10	−0.09
		Snowella	−0.02	**−0.61****	−0.13
	Oscillatoriales	*Microcoleus*	0.26	−0.17	**−0.43***
		Oscillatoria	**0.31**	−0.14	**−0.33**
		Phormidium	**0.31**	−0.14	**−0.43***
		Planktothrix	0.27	−0.10	**−0.45***
	Pseudanabaenales	*Leptolyngbya*	0.08	**−0.50****	−0.17
		Limnothrix	0.12	−0.24	−0.28
		Prochlorothrix	−0.10	**−0.62****	−0.03
		Pseudanabaena	0.03	−0.09	−0.45*
	Nostocales	*Aphanizomenon*	−0.09	−0.21	−0.33
		Calothrix	0.24	−0.18	−0.43*
		Cylindrospermopsis	−0.08	**−0.33**	−0.42*
		Dolichospermum	−0.05	0.16	−0.27
		Nostoc	0.26	0.04	**−0.53****
Actinobacteria	Actinomycetales	*Arcanobacterium*	−0.12	**0.70****	0.08
		Cryobacterium	−0.13	**0.41***	0.09
		Demequina	0.07	**0.59****	0.23
		Georgenia	−0.10	**0.58****	0.21
		Mycobacterium	0.16	−0.20	0.29
		Nocardia	0.04	**−0.63****	0.18
		Rhodococcus	0.15	**0.71****	−0.18
		Saccharomonospora	0.05	**0.49****	**0.32**
		Saccharopolyspora	**0.33**	**0.59****	**−0.35**
		Sanguibacter	0.07	**0.58****	0.20
		Streptomyces	−0.12	0.00	**0.45***
		Streptosporangium	0.25	**−0.35**	0.11
Metabolites		MIB	−0.09	−0.22	**0.45***
		GSM	0.16	−0.29	−0.08

Note. Correlations with strong statistical significance are in bold $p<0.05$. GSM = geosmin, MIB = 2-methylisoborneol.
*$p<0.01$. **$p<0.001$.

Australian reservoir showed that occurrences of MIB were linked to increasing ammonia concentrations in water (Uwins et al., 2007). In the present study, although GSM showed no correlation to inorganic nitrogen, MIB levels are strongly linked to ammonia in water ($p < 0.01$; Table 4) consistent with the observations of Uwins et al. (2007).

3.7.2. Actinobacteria

These bacteria have shown a positive correlation between nitrogen concentration and production of odorous metabolites (Lind & Katzif, 1988). In Eagle Creek Reservoir, most Actinobacteria (Table 4) are strongly correlated to high levels of nitrate (*Arcanobacterium*, $p < 0.001$; *Demequina*, $p < 0.001$; *Rhodococcus*, $p < 0.001$) and to ammonia (*Saccharomonospora*, $p < 0.05$; *Streptomyces*, $p < 0.01$). This supports the CCA results (Figure 5) that these actinobacterial genera occurred during high discharge periods in spring 2013 when nitrogen concentrations were maximal. While inorganic nitrogen may promote the growth of many Actinobacteria, *Streptomyces*, a potent producer of MIB (Saadoun et al., 1997), is the only actinobacterial genus in Eagle Creek Reservoir that shows a concurrent positive correlation to NH_3 (Table 4; $\rho = 0.45$, $p < 0.01$) and to MIB occurrences (Table 3; $\rho = 0.42$, $p < 0.01$). Although *Saccharomonospora* has a similar profile as *Streptomyces*, its growth is more likely due to the presence of high NO_3^- ($p < 0.001$) in water rather than NH_3 ($p < 0.05$). Additionally, its own MIB biosynthesis capacity has never been demonstrated.

3.7.3. Cyanobacteria

Conversely to Actinobacteria, the majority of Cyanobacteria are negatively correlated to nitrate and ammonia levels in the reservoir water (Table 4). Negative correlations are explained by the fact that most Cyanobacteria thrived in the epilimnion during the summer stratification when nitrogen was depleted.

Nonheterocystous (Oscillatoriales and Pseudanabaenales) and heterocystous (Nostocales) Cyanobacteria also have the capacity to fix atmospheric nitrogen (Bergman et al., 1997; Fay, 1992) and do not exclusively rely on the reservoir's nitrogen availability. However, Spearman's correlations highlight significant relationships between nitrite and Oscillatoriales (*Oscillatoria* and *Phormidium*; $p < 0.05$). Although statistically nonsignificant, nitrite seems to influence the growth of potential GSM-producing bacteria such as *Microcoleus* ($p = 0.09$), *Planktothrix* ($p = 0.08$), and *Nostoc* ($p = 0.09$). The importance of nitrite during odorous outbreaks in Eagle Creek Reservoir is also suggested from the CCA results in Figure 5.

4. Conclusions

In Eagle Creek Reservoir, recurring major T&O episodes are very frequently observed during the spring and the fall, while fewer detections are recorded during the summer time. These odorous events usually occur after the reservoir has received inflows from its main tributary in April and May. Spring outbreaks of MIB and GSM have in general longer durations and are more intense than any other times later in the year. High stream discharges bring in nutrients and mix the reservoir water columns. These conditions are favorable to support the growth of some Actinobacteria (*Streptomyces*) and Cyanobacteria (*Planktothrix*) that are involved in the in situ production of MIB and GSM. In the present study, a lag phase of 37 days between a major peak discharge and highest detections of both metabolites in the reservoir waters was observed. This lag phase seems to represent the time required for Actinobacteria to be transported from the watershed to the reservoir. This information provides a useful clue for managers desiring to anticipate major odorous events and may want to disrupt the bacterial growth before it becomes severe. GSM was strongly linked to the presence of *Planktothrix* in the reservoir while MIB detections frequently occurred when *Streptomyces* was around. As seen in Eagle Creek Reservoir, besides a few fathead minnow kills, the algaecide treatment was only effective against the decaying bloom of Cyanobacteria and disrupted the GSM production, whereas it had little impact on copper-resistant Actinobacteria and MIB, which remained detectable in the water a couple of weeks after the treatment. This observation highlights a difference in the chemical and biological behavior of the two metabolites MIB and GSM, which should influence the choice of decision makers before treating a water supply reservoir. Furthermore, differences in the spatial distribution of MIB and GSM noticed in this study can be valuable for water utilities that have the ability to modify their water uptake depths. While some GSM production occurs in the epilimnion, hypolimnetic MIB levels during the summer stratification were more elevated than in the upper layers. Any uptake from bottom waters must be avoided as there is a potential risk for MIB contamination. Genetics remains an important tool while studying bacterial communities in aquatic environments. The 16S rRNA gene sequencing method can provide key insights regarding the presence of potential T&O-producing bacteria at a given time compared to traditional morphologically based microscope counting techniques that can miss information about species without morphological distinctiveness, such as the Actinobacteria.

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